AD_____

Award Number: W81XWH-07-2-0082

TITLE: GEIS Surveillance Network Program

PRINCIPAL INVESTIGATOR: COL Rodney Coldren

CONTRACTING ORGANIZATION: Kenya Medical Research Institute
Nairobi 00200 Kenya

REPORT DATE: October 2013

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE
October 2013

2. REPORT TYPE
Annual

3. DATES COVERED
13 September 2012 – 12 September 2013

4. TITLE AND SUBTITLE
GEIS Surveillance Network Program

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-07-2-0082

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
COL Rodney L Coldren

E-Mail: Rodney.coldren@usamru-k.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Kenya Medical Research Isntitute
Plot Line Off Mbagathi Rd
Nairobi 00200
Kenya

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The purpose of this contract is to carry out emerging infectious disease surveillance in Kenya. Specific areas in which work is performed include respiratory illness surveillance (particularly influenza), acute febrile illness surveillance, malaria resistance surveillance, diarrhea etiology and antimicrobial resistance surveillance, sexually transmitted illness surveillance, and capacity building. KEMRI maintained surveillance sites in both Kenyan Defense Forces and Ministry of Health clinics and hospitals throughout Kenya. KEMRI operated reference laboratories for this work in Nairobi, Kericho, and Kisumu, including the National Influenza Center (NIC), the arbovirus reference laboratory, the antimalarial resistance laboratory, entomology facilities, the Center of Excellence in Microscopy, the microbiology reference laboratory. Capacity development projects include laboratory renovations in one military clinic (Moi Barracks) and one civilian clinic (Port Reitz District Hospital) and continuation of a laboratory and medical maintenance student attachment program. The program was able to characterize respiratory viruses causing influenza-like illness in Kenya, determine etiologies of diarrheal illnesses and the antimicrobial resistance patterns of bacterial causes, determine the etiologies of sexually transmitted infections and acute febrile illnesses in military and civilian populations, and establish the pattern of antimalarial resistance across Kenya.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	14	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

INTRODUCTION:

KEMRI supports USAMRU-K's establishment of an emerging infectious disease surveillance network by providing contract personnel, laboratory and administrative facilities, capacity development capabilities for contracted personnel and partner organizations, regulatory oversight, and other required functions for the performance of infectious disease surveillance and research. The areas of research/surveillance performed are categorized by the pillars as defined by the US Department of Defense's Armed Forces Health Surveillance Center Department of Global Emerging Infectious Disease Surveillance and Response (DoD-GEIS). These pillars include respiratory illnesses, acute febrile illnesses, malaria, enterics, sexually transmitted infections and antimicrobial resistance, and capacity building. KEMRI maintains both surveillance sites and central laboratories to accomplish this mission.

BODY: For clarity's sake, this report will be divided by DoD-GEIS pillar.

Respiratory Illness:

Global influenza surveillance to detect viral antigenic drifts and shifts must be reliably undertaken to protect public health. Sub-Saharan African countries have lacked laboratories and programs to conduct sustained influenza surveillance. To address this problem, USAMRU-K-GEIS and KEMRI developed a human influenza sentinel surveillance program at 8 civilian hospitals and 1Kenya military hospital since 2006. Influenza diagnostics is undertaken at the National Influenza Center (NIC) developed intoa state-of-the-art BSL-2 laboratory through a significant monetary investment by GEIS. KEMRI also plays a significant role in assisting Kenya in Influenza Pandemic and other outbreak response.

Between October 1st and December 31st 2012, 333 NP swab specimens were received and processed at the NIC from ILI patients from the regular USAMRU-K sentinel surveillance network. In addition, 13 NP swab specimens were received and processed from SARI patients during the same period. During the 1st quarter of FY13, 75 (22.5%) of all specimens collected from ILI patients tested positive for influenza by RT PCR. Of these, 49 (65%) were influenza A and 26 (35%) were influenza B¿s. When the 49 influenza A positive sample were subtyped using PCR, all (100%) were found to be A(H3N2) subtype. No influenza A (H1N1pdm09) or A(H1N1) subtypes were detected. Seasonal A(H1N1) cases were last seen in Kenya in November 2009. Of the 49 influenza A PCR-positive samples, influenza virus isolates were obtained from only 7 samples after the samples were inoculated in MDCK cells. All the 7 influenza A isolates were A(H3N2). 31 samples that tested positive for influenza A(H3N2) by PCR did not produce CPE upon inoculation in MDCK. Likewise of the 26 influenza B PCR-positive samples, influenza viruses were obtained from 12 samples and the other 14 did not yield virus isolates. Amongst the 13 SARI cases from whom NP swabs were obtained, two cases tested positive for an influenza virus by PCR. One case had influenza B virus whereas the other case had influenza A(H3N2) virus. The influenza B case was a 6 month old female patient whereas the influenza A(H3N2) case was a 3 months old male. The admission diagnoses for the two patients were bronchiopneumonia and acute severe pneumonia respectively. 81 non-influenza respiratory viruses were isolated through inoculation of NP samples in appropriate cell lines. These included Adenoviruses (16%), RSV (12.3 %), Enteroviruses (18.5%), Parainfluenzavirus type 1 (21%), Parainfluenzavirus type 2 (16%) and Parainfluenzavirus type 3 (16%). We sequenced 135 gene segments at the NIC. 108 of these were from our regular influenza surveillance activities and consisted of 24 influenza A (H3N2) full gene segments comprising 8 M, HA and NA gene segments. 84 were influenza B full gene segments comprising of NA, M, and HA derived from 28 isolates. The nucleotide sequences of the all the influenza A viruse gene segments and 24 gene segments from 8 Flu B¿s have been shared with USAFSAM for inclusion in the global VRBPAC flu sequence analysis. These have also been deposited in GenBank or GISAID.

Between January 1st and March 31st 2013, 235 NP swab specimens were received and processed at the NIC from ILI patients from the regular USAMRU-K sentinel surveillance network. In addition, 40 NP swab specimens were received from SARI patients and processed during the same period. During the 2nd quarter of FY13, 44 (19%) of all specimens collected from ILI patients tested positive for influenza by RT PCR. Of these, 40 (91%) were influenza A and 4 (9%) were influenza B's. When the 40 influenza A positive sample were subtyped using PCR, 25 (62.5%) were found to be A(H3N2) and 15 (37.5%) we influenza A (H1N1pdm09). No seasonal A(H1N1) subtype viruses were detected. Seasonal A(H1N1) cases were last seen in Kenya in November 2009. Thus, just like the second and third and fourth quarters of FY2012 and first quarter of FY13, seasonal influenza A (H3N2) was the predominant subtype circulating in Kenya in the second quarter of FY2013. Of the 40 influenza A PCR-positive samples, only 36 samples were inoculated in MDCK cells and results obtained. Of these 23 were A(H3N2) and 13 were (H1N1)pdm09. 8 (34.8% isolation rate) A(H3N2) isolates and 10 (76.9% isolation rate) A(H1N1)pdm09 were obtained. In addition, one influenza B isolate was inoculated in MDCK cells and yielded a single isolate. 2 A(H1N1)pdm09, 2 seasonal A(H3N2) and 3 Influenza B samples are pending, awaiting inoculation in MDCK cells. These will be reported in the next quarter. Amongst the 40 SARI cases from whom NP swabs were obtained, four (10%) cases tested positive for an influenza virus by PCR. Two case had A(H1N1)pdm09 virus whereas the other two case had influenza A(H3N2) virus. The A(H1N1)pdm09 cases were from a 1yr 9moths (male) and a 1yr 3months (female) children. The admission diagnoses for the two patients were severe pneumonia. The A(H3N2) cases were from a 1 yr 3moths and a 1yr 9moths old patients. Both patients were female. The admission diagnosis for the two patients was severe pneumonia. In the second quarter of FY13, 32 non-influenza respiratory viruses were isolated through inoculation of NP samples in appropriate cell lines. 24 of these were from the ILI surveillance, whereas the other 8 were from SARI cases. Analyses of the samples yielded the following non-influenza isolates: one HSV1, four Enteroviruses, two Parainfluenza2, two RSV, four Adenovirus, four Parainfluenza3 and seven Parainfluenza1. Among the SARI cases, these included one Enterovirus, five RSV, one Parainfluenza3 and one Adenovirus.

Between April 1st and June 30th 2013, 388 NP swab specimens were received and processed at the NIC from ILI patients from the regular USAMRU-K sentinel surveillance network. In addition, 56 NP swab specimens were received from SARI patients and processed during the same period. 42 (10.8%) of all specimens collected from ILI patients tested positive for influenza by RT PCR. Of these, 29 (69%) were influenza A and 13 (31%) were influenza B's. When the 29 influenza A positive sample were sub-typed using PCR, 15 (52%) were found to be A(H3N2) and 14 (48%) we influenza A (H1N1)pdm09. No seasonal A(H1N1) subtype viruses were detected. Seasonal A(H1N1) cases were last seen in Kenya in November 2009. Thus, just like the second and third and fourth quarters of FY2012, first and second quarters of FY13, seasonal influenza A (H3N2) was the predominant subtype circulating in Kenya in the third quarter of FY2013. Of the 29 influenza A PCR-positive samples, only 21 [8 A(H3N2) and 13 (H1N1)pdm09] samples were inoculated in MDCK cells and results obtained. The results yielded isolates for 1 A(H3N2) (12.5% isolation rate) and 9 (69.2% isolation rate) A(H1N1)pdm09. 8 PCR-positive samples consisting of 7 A(H3N2) and 1 A(H1N1)pdm09 are pending inoculation in MDCK cells and will be reported in the next quarter. Of the 13 samples positive for influenza B by PCR, 11 were inoculated in MDCK and 2 are pending and will be reported in the next quarterly report. Out of the patient samples testing positive for 11 influenza B by PCR, only 6 isolates were obtained. These include 1 B/Brisbane/60/2008-like (B/Victoria lineage) and 5 B/Wisconsin/1/2010-like (B/Yamagata lineage). Amongst the 56 SARI cases from which NP swabs were obtained, 1 case tested positive for an influenza B virus by PCR. An influenza B virus was isolated from this sample and confirmed to be B/Wisconsin/1/2010-like (B/Yamagata lineage). This sample was obtained from a female 5 yrs and 6 months child admitted to Mbagathi district hospital with

severe pneumonia. In the second quarter of FY13, 135 non-influenza respiratory viruses were isolated through inoculation of NP samples in appropriate cell lines. 125 of these were from the ILI surveillance, whereas the other 10 were from SARI cases. Analyses of the samples yielded the following non-influenza isolates: 12 enteroviruses (11 from ILI and 1from SARI patients), 28 Parainfluenza2 (25 from ILI and 3 from SARI patients), 23 RSV (19 from ILI and 4 from SARI patients), 5 Adenovirus (all from ILI patients), 19 Parainfluenza 3 (all from ILI patients) and 32 Parainfluenza 1 (30 from ILI and 2 from SARI patients).

Between July 1st and September 30th 2013, we received 332 NP swab specimens at the central laboratory from ILI patients participating in the regular USAMRU-K sentinel surveillance network. Amongst these 322 were processed and tested for presence of influenza viruses and 10 are pending analysis. In addition, 69 NP swab specimens were received from SARI patients and processed for detection of respiratory viruses during the same period.
In the 3rd quarter report of FY13 we reported that 8 influenza A PCR-positive samples consisting of 7 A(H3N2) & 1 A(H1N1)pdm09 as well as 2 influenza B positive samples were pending, awaiting viruses isolation by inoculation in MDCK cells and that these results would be reported this quarter. All these samples had real time PCR Ct values above 30 indicating ow viral loads in the patients. Upon inoculation in MDCK cells, none of the samples showed CPE after a full incubation period of fourteen days in MDCK cells. This was not surprising because in the real time PCR assay, it is often difficult to isolate influenza viruses from samples that show high Ct values due to the low virus loads in these samples. 62 (19.3%) of the specimens collected from ILI patients tested positive for influenza by real time RT PCR. Of these, 37 (59.7%) were influenza A and 25 (40.3%) were influenza B's. No influenza C viruses were seen during this period. When the 37 influenza A positive sample were sub-typed using PCR, 30 (81%) were found to be A(H3N2) and 7 (19%) were influenza A (H1N1)pdm09. No seasonal A(H1N1) subtype viruses were detected. Seasonal A(H1N1) cases were last seen in Kenya in November 2009. Thus, just like the second and third and fourth quarters of FY2012 and the first three quarters of FY13, seasonal influenza A (H3N2) was the predominant subtype circulating in Kenya in the fourth quarter of FY2013. Of the 37 influenza A PCR-positive samples, only 35 [28 A(H3N2) and 7 (H1N1)pdm09] samples were inoculated in MDCK cells. The results yielded isolates for 3 A(H3N2) (10.7% isolation rate) and 3 (42.9% isolation rate) of A(H1N1)pdm09. The isolation rate of 10.7% for A(H3N2) is very low. Whereas inoculation in MDCK-SIAT1 cells, engineered to express increased levels of α-2,6-linked sialic acid receptors would improve this isolation rates in these patient samples (Oh et al., 2008), we do not have access to this cell line to improve our isolation rates. Of the 25 samples positive for influenza B by PCR, 22 were inoculated in MDCK and 3 are pending and will be reported in the next quarterly report. Out of the 22 patient samples testing positive for influenza B by PCR, 20 isolates were obtained and 2 did not yield isolates. Thus the isolation rate for influenza B during this quarter was 90.9%. HAI results showed that all the influenza B isolates obtained in this quarter were B/Wisconsin/1/2010-like (B/Yamagata lineage). Amongst the 69 SARI cases from which NP swabs were obtained, 1 case tested positive for an influenza B virus by PCR. This influenza B virus isolate was confirmed to be B/Wisconsin/1/2010-like (B/Yamagata lineage). This sample was obtained from a 1 yr and 8 months male baby admitted to New Nyanza Provincial hospital with very severe pneumonia but was discharged after hospitalization for three days. 34 non-influenza respiratory viruses were isolated through inoculation of NP samples in appropriate cell lines. 33 of these were from the ILI surveillance network, whereas the 1 was from a SARI case. Analyses of the samples yielded the following non-influenza isolates: 9 enteroviruses (all from ILI patients), 2 parainfluenza2 (both from ILI patients), 10 Adenovirus (all from ILI patients), 4 parainfluenza 3 (3 from ILI and 1 from SARI patients) and 9 Parainfluenza 1(all from patients).

Because of changes in funding, this contract will no longer support the National Influenza Center nor collection from civilian sites in the coming year (beginning 1 Oct 2013). Collection will continue at military sites and all other work will remain unchanged.

Acute Febrile Illness:

The project continued to play a key role in surveillance work for pathogens associated with febrile illnesses, outbreak response and training of MOH personnel. A cumulative total of 3534 samples have been collected and inventoried. Of these, 3252 have been analyzed for malaria, leishmania, dengue, leptospirosis, rickettsiae, salmonella, brucella spp, Q-fever, Erlichia, human anaplasmosis, borellia. The presenting signsfor these illness were non-specific comprising of chills, headache, running nose, abdominal pain, vomiting, diarrhea, joint pains, general malaise. Of the samples analyzed, only 53.7% had an associated etiology: Malaria (47.0%, EBV (39.7%, salmonella (19.9%, Q-Fever (10.5%, rickettsia (5.0%), Dengue (2.8%), Leptospira (0.4%) and brucella (0.4%. Most of the infections were mono infections (41.6%: Malaria (40.4%), EBV (32.7%), Q-fever (7.2%, salmonella (14.4%), rickettsia (3.3%), brucella (0.3%) and Dengue (3.1%). Only 11.1% of the samples had two pathogens and 1.3% with three pathogens. Of the co-infections, malaria occurred with EBV at 31.9%, salmonella at 14.4%, Q-fever at 12.6%, Rickettsia at 6.3% and dengue at 1.4% and with leptospira at 0.4%. 45.7% of fevers had no etiology, most coming from the Kisii highlands (36%), Coast (31%), Lake Basin (19%), and arid/ semi arid region (8%). Figures 1, 2 and 3 summarizes these datasets. From these observations we conclude that, in the areas of study, most blood borne febrile illnesses are mono infections of malaria, EBV, salmonella and Q-fever and that, robust platforms will be required for unbiased pathogen detection/discovery, especially for close to 50% of fevers without an etiology.

The Entomology team trained a total 5 field workers in the three sites who were equipped with expertise to conduct sampling once per week using CDC light traps baited with dry ice in three chosen locations. For each site, 4 standard CDC light trap was used for surveillance. Two field workers were trained per site in Lodwar and Gilgil while one was trained in Marigat since we already have a staff stationed at the Marigat field station. During the training, sand flies sampling was done for consecutive 3 nights using 4 CDC, dry ice baited light traps. In Marigat, sampling continued through September. Routine sampling in Gilgil and Lodwar is yet to be implemented due to logistical constraints. Sampled sand flies were stored in cryovials containing 70 % ethanol and labeled according to site, date of collection and trap number, and later shipped to Kisian entomology laboratory where they were dissected, cleared, mounted and identified to species. The female abdomens were preserved in -80 for future leishmania testing assay.

Fifty seven clinical samples from Mombasa were screened for Dengue virus, fourteen tested positive. A total of nineteen Dengue positive samples were serotyped (five Dengue positive samples that were pending serotyping in the month of June and the fourteen Dengue virus positives); 11 were Dengue virus type 1, and 8 were Dengue virus type 2. A total of 23 Mosquito pool passages were tested for Dengue virus, none was positive. In August Twenty Four clinical samples (22 from Mombasa, 1 from AIC Kijabe Hospital and 1 from Centre for Tropical and Travel Medicine) were screened for Dengue virus, five tested positive. The Dengue positive samples were serotyped; 2 were Dengue virus type 1, and 3 were Dengue virus type 2. A total of 140 passages (47 Mosquito pools and 93 clinical samples) were tested for Dengue virus and 16 clinical samples tested positive while all the mosquito passages were negative. On serotyping, 8 were DENV 1 and 5 were DENV 2, three were untypable. One mosquito isolate from Baringo was tested for Flaviviruses, Rift Valley Fever virus, Alphaviruses and orthobunyaviruses and it tested positive for orthobunyavirus (Bunyamwera virus specifically). In September a total of Six clinical samples (2 from a Nairobi hospital, 1 from AAR health and 3 from Amisom Level II Field Hospital) were screened. Two samples (one from a Nairobi hospital and 1from AAR) were tested for Flavivirus and YF virus, both tested negative for the two tests. One sample (from a Nairobi hospital) was tested for Flavivirus, Orthobunyavirus, Alphavirus, RVFV and Filoviruses, and it tested negative for all the tests. The

3 AMISOM samples were tested for Flavivirus, Orthobunyavirus, Alphavirus, RVFV, Filoviruses, CCCHFV, Hepatitis A and E and they tested negative for all. A total of 4 mosquito isolates (2 from Baringo, I from Tanadelta and 1 from Garissa) were tested for Flavivirus, Orthobunyavirus, Alphavirus, and RVFV. Two from Baringo tested positive for Orthobunyavirus (Bunyamwera virus specifically) and the other two (one each from Tana delta and Garissa) tested positive for Alphavirus (Ndumu virus specifically).

Leishmaniasis is a neglected tropical disease caused by protozoan parasites of the Leishmania genus, and transmitted by sandfly bites from about 30 species that are proven vectors. There are three forms of leishmaniasis: visceral (VL), cutaneous (CL), and mucosal (ML), of which VL is the most severe. In leishmania endemic areas, risk to leishmaniasis is highest in children as they have not yet developed immunity to the disease. Many adults in those situations are reservoirs, facilitating continuing disease transmission to those without immunity. In Kenya, leishmania risk is poorly mapped. Following identification of human cases of VL in Marigat, Gilgil and Lodwar, three sand flies surveys were conducted at these sites by USAMRU-K/KEMRI entomology teams. In addition, because of the increasing travel restrictions, we have opted to train residents of these sites on methods of trapping sand flies. In this regard, the Entomology team trained a total 5 field workers at the three sites on the methods of sampling once per week using CDC light traps baited with dry ice. For each site, 4 standard CDC light traps were used for surveillance. Two field workers were trained at each site, while one was trained in Marigat since we already have a staff stationed at the Marigat field station. During the training, sand flies sampling was done for 3 consecutive. In Marigat, sampling continued through September. Routine sampling in Gilgil and Lodwar is yet to be implemented due to logistical constraints. Sampled sand flies were stored in cryovials containing 70 % ethanol and labeled according to site, date of collection and trap number, and later shipped to Kisian entomology laboratory where they were dissected, mounted and identified to species level. The female abdomens were preserved in -80 for future leishmania testing assays. In addition 2 individuals from Wajir District Hospital, located near the border of Kenya and Somalia were trained on the leishmania case detection and surveillance. Wajir has had multiple outbreaks of VL and the training will allow close monitoring of outbreaks, detection, diagnosis and treatment by MOH personnel. 1.3% of individual with AFI have been found to have VL. MOH personnel at affected sites have been informed.

Malaria:

P. falciparum (Pf) drug resistance, conferred largely by mutations, is expected to continue evolving in Kenya. Information from in vitro Pf malaria drug resistance patterns and molecular mutations can is critical in tracking new patterns of emerging or disappearing and can be used in selecting effective malaria drugs. The SYBR Green I-based IC50 drug sensitivity assay is used for ex vivo and in vitro drug sensitivity testing. In vitro drug sensitivity testing is done using the newly installed liquid handler, drug testing platform, Biomek FXp. Testing was done against 2 index Pf clones [chloroquine (CQ)-sensitive (D6), CQ-resistant (W2)]. Twelve drugs were tested in the standard panel which includes chloroquine, quinine, artemisinin, amodiaquine, artemether, lumefantrine, atovaquone, tafenoquine, dihydroartemisinin, Piperaquine, Mefloquine and doxycycline. Additional drugs were tested in the extended panel depending on the availability of the blood sample. These drugs include Arteether, Pyronaridine, Primaquine, Artesunate, Proguanil, Trimethoprim, Sulfamethoxazole, Pyrimethamine and Sulfadoxine. Molecular analysis is currently done using conventional PCR, real-time PCR, Capillary Electrophoresis, Allelic discrimination assays and Sanger sequencing. Genes analyzed include Pfmdr-1, Pfcrt, Pfdhfr, Pfdhps, Pfmrp, Pfcytb, Pfnhe-1, pfmdr-6, Pfmdt and Pftetq. These genes are linked to resistance of different drugs that are found in our in vitro test panels.

A total of 610 sample isolates were collected, representing a 72% increase in enrolment compared to the previous year. Samples from KDH and KBW, approximately 15 minutes drive from the lab were assayed by “immediate ex vivo” whereas those from other sites which are far from the MDR labs in Kisian were tested using the in vitro technique. These samples were tested along with 2 reference Pf clones [chloroquine (CQ)-sensitive (D6), CQ-resistant (W2)] which were first cryopreserved for culture to adapt to in vitro replication prior to testing. Drugs tested include chloroquine (CQ), quinine (QN), artemisinin (AR), amodiaquine (AQ), artemether (AT), lumefantrine (LU), atovaquone (AV), tafenoquine (TQ), dihydroartemisinin (DHA), Piperaquine (PPQ), Mefloquine (MQ) and doxycycline (DX). DHA and PPQ were additional drugs included to drug panel due to priority changes by the Kenya Ministries of Health and the scientific/public health consensus of intended use of Duo Cotecxin in place of coartem in future. The number of drugs assayed was doubled from 12 to 24 for the 4th quarter. The additional drugs included four on the standard drug panel:- halofantrine, primaquine, arteether, artesunate; eight anti-folate drugs namely:- pyronaridine, pyrimethamine, dapsone, trimethoprine, sulfamethoxazole, chlorcycloguanil, sulfadoxine, proguanil. In the 4th quarter, a total of 139 isolates were successfully assayed. Drug susceptibility profiles for 16 drugs, expressed as 50% inhibition concentration (IC50s) were conducted. For FY14, 194 isolates were assayed successfully.

Malaria mortality and morbidity remains high in sub-Saharan Africa, especially in HIV-infected individuals who are more likely to develop malaria disease than those uninfected. HIV-infected individuals frequently contract opportunistic bacterial infections that are controlled with Trimethoprim/sulfamethoxazole (TMP/SMZ). Use of TMP/SMZ prophylaxis however might put populations at risk of developing significant cross-resistance to closely related antimalarials such as antifolate. The need for daily use of TMP/SMZ following the initiation of antiretroviral therapy (ART) and resultant immune reconstitution has been questioned. Most importantly, the effect of discontinuing TMP/SMZ prophylaxis on malaria is likely to drive policy recommendations. A surveillance study will be conducted to evaluate impact of antibiotics and antiretroviral use on the genotypic and phenotypic drug resistance profiles of Plasmodium falciparum found in subjects randomized to continue vs. discontinue TMP/SMZ prophylaxis. This information is critical to malaria and HIV treatment to DoD and policy makers as strategies are mapped out to prioritize DoD product development for the war fighter and contain the effects of these two diseases. The study protocol was approved. The clinical trial study was completed and closed out. More than 4000 specimen were collected. The samples have not yet been moved from the freezers in Nairobi to Kisumu but it is expected by year end, the samples will be in Kisumu. Two people assigned to this project have now extensively been trained and are now proficient with most of the assays. They have started processing samples from other study sites.

Microscopy remains the standard method for malaria diagnosis. Evolving methods such as rapid tests have been introduced to augment diagnosis where reliable laboratory services are not available or feasible. Limitations of these methods in clinical and research settings are well documented. This heightens the risk of misdiagnosis for US soldiers serving in endemic areas as well as failure of research studies conducted by US DOD facilities. It therefore important that laboratory personnel involved in malaria diagnosis be trained in proper microscopic diagnosis and relevant quality systems in order to improve reliability of results. This should be followed by provision of external quality assurance and supportive supervision to ensure that skills attained and standards established are maintained. These can only be done if reference malaria blood films are made available. Evolving methods such as rapid tests and related molecular assays need evaluation using characterized specimen such as whole blood, dried blood spots and tubes. These are useful for both laboratory and field based evaluations. 150 volunteers for malaria by microscopy, 50 tested positive., 46 with P. falciparum and 4 mixed infections of P. falciparum and P. malariae. Of the 50 cases, 15 P. falciparum and 3 of the P. falciparum and P. malariae mixed infections cases were enrolled for

additional blood draw. From these, approximately 5000 blood films and 300 whole blood aliquots were prepared for microscopy training and evaluation of alternative malaria diagnostic tests. 38 Kenya MOH laboratory personnel were trained on malaria microscopy. Observed pre and post-training sensitivity was 61%-81%, specificity 82% -89%, species identification 51%-81% and parasite counting 65% - 78%. Proficiency in microscopic diagnosis of malaria was conducted for 28 Kenya MOH laboratory personnel facilitating implementation of quality assurance plan for public health facilities. Average sensitivity was 91%, specificity 92%, and species identification 72%. Composite accuracy was 86%.

Enterics:

Acute gastroenteritis is a debilitating disease and is considered a major disease nonbattle injury for deployed U.S. military personnel. A clinical surveillance protocol (WRAIR #1549) to identify microbial pathogens from human stool specimens collected at sites within the GEIS network in Kenya is currently being conducted at the Microbiology Hub Kericho (MHK), USAMRU-Kenya/KEMRI. This protocol is a case (volunteers with acute diarrhea) control (volunteers with no diarrhea) study that allows for the collection of stool specimens recruited at an outpatient clinical setting. Briefly, stool specimens are collected in preservation media at the surveillance sites and transported to the MHK where they are processed and tested for bacterial, parasitic, and viral pathogens. Enteric bacteria identification and antibiotic susceptibility are conducted. Ova and cysts of parasites are identified by general and immunofluorescence microscopy. Enteric viruses are diagnosed using either an enzyme immunoassay or PCR. The greatest benefit to the DoD is having a highly competent microbial disease clinical laboratory that will provide much needed support to the AFRICOM mission. Scientifically, the enterics surveillance conducted is providing valuable data on the prevalence of enteric pathogens in Kenya as well as potential patterns of antibiotic resistance among bacterial isolates in Kenya. Year to date closeout totals are 978 stool samples collected and tested by the MHK. Pathogen identifications are recorded in the attached spreadsheets. Isiolo District Hospital located in Isiolo was activated as a site on 30 July 2012 and Kisumu District West located in Kisumu was opened for collection December 2012.The laboratory for the Kenyan Defense Force Eldoret site was renovated in the 4th quarter of FY12 and the amendment received approval at KEMRI Q2 FY13. KDF will be activated for sample collection pending WRAIR IRB approval; this was achieved 3rd quarter 2013. The Microbiology Hub Kericho maintains College of American Pathologists (CAP) certification.

569 samples were collected during the fiscal year in children under 5yo. Of these, 284 were cases and 285 were controls. Cases were more likely to be positive for campylobacter sp (OR 1.5), shigella sp (OR 3.1), astrovirus (OR 3.1), and rotavirus (OR 3.7). Cases were less likely to demonstrate norovirus (OR 0.55). The most common parasites identified were blastocystis hominis and entamoeba coli.

409 samples were collected in individuals over the age of 5yo. Of these, 204 were cases and 205 were controls. Cases were more likely to be positive for shigella sp (OR 4.8) and less likely to be positive for astroviruses (OR 0.45). The most common parasites identified again were blastocystis hominis and entamoeba coli.

Sexually Transmitted Infections:

In Kenya, one of the more prosperous countries in East Africa, patients presenting to Ministry of Heath clinics with complaints suggestive of STIs (discharge or genital ulcer) often go undiagnosed, and are treated empirically with broad spectrum antibiotics. The drug resistance profiles, especially of gonorrhea, is largely unknown. In partnership with MOH and the KDF, all patients presenting to Kisumu and the Mbagathi District Hospital, the Mutwonge Naval base, Lanet Military barracks clinic and Kahawa barracks clinic with symptoms suggestive of gonorrhoea are offered anonymous screening for gonorrhea and chlamydia

(GC). Specimens are taken for detection and isolation of Neisseria gonorrhoeae. Treatment is provided as per the ministry of Public Health and Sanitation guidelines. Antimicrobial susceptibility is determined using the E test method. 84 samples were tested of which 34 were positive for GC, 5 for Chlamydia, and 3 for both. Specimens will be transported to the Uniformed Services University of the Health Sciences for further testing.

Capacity Development:

KEMRI/Walter Reed Project - Kisumu operates a Health and Demographic Surveillance System (HDSS) in Seme Sub County and Parts of Kisumu Sub-County (under the new devolved structure). The program is designed to track the evolving health status and demographics of the study population over time and to detect and signal if there is an outbreak or emergence of a new disease. This platform offers tremendous capability to conduct population-based surveillance at the individual, household/compound and community level. Already, three protocols for surveillance of specific infectious diseases in this community setting (rotavirus, influenza, and tuberculosis) have been designed. The Kisumu West DSS recently became a full member of the INDEPTH network (http://www.indepth-network.org/) and is continuing to grow and mature into a sustainable platform collecting high quality data. The program has added a strong health surveying component and is currently conducting routine bi-annual surveying of every household. With the expansion of the program (Both in terms of relevance and complexity), our staff require a higher level training and education in mapping techniques, data management, statistical analysis as well as attendance at relevant seminars to confer and expand our collaboration with local and international colleagues. Currently, the HDSS program shares aggregate demographic data with local MOH and PEPFAR leadership to inform programs and interventions, but this link is further set to be expanded and strengthened. The program is currently conducting the third round of Bi-annual household surveys. A population of 143,273 individuals drawn from 27,879 households is currently being monitored for demographic (Births, Deaths, Migrations) and Health (Causes of Morbidity) changes. Information on pregnancy as well as Verbal Autopsy interviews (to determine causes of death on reported deaths) is also being collected. The surveys are set to be completed in Mid November 2013. One staff has been selected for a fully sponsored course on Social Determinants of Health (SDH) by the INTREC program (INDEPTH training and research centers of excellence. http://www.intrec.info/courses.html. The entire program consists of a series of components including an online course, quantitative and qualitative workshops in Accra (Ghana) and hands-on workshop on data analyses at Harvard University. The program will conclude in fall 2014 with presentations of the research training results, written as publishable papers for international journals A total of 855 Verbal Autopsy interviews have been conducted (out of the 1160 reported deaths during the first round of survey). The information will provide crucial cause of death data important for conducting research and public health interventions in the study area. A GIS refresher course was held in Kisumu (2nd September 2013) by a representative from ESRI East Africa (http://www.esriea.co.ke/). 6 staff from KWHDSS attended the session which was held at the Kombewa Clinical research center.

The student attachment program emphasizes hands on training experience for students in a professional environment. All interns undergo laboratory rotation in three laboratories in Nairobi handling arboviruses, respiratory viruses and sexually transmitted infections (STI). The training schedule comprises of three months training in specialized laboratory techniques used in the identification and characterization of emerging and re-emerging infectious diseases under the supervision of technical staff and scientists. During their internship students also get trained on laboratory safety, biosecurity, quality assurance and quality control. The program ensures that students' progress is systematically monitored and that student support systems are available. The internship program provides undergraduates and diploma students' practical training and research experiences to strengthen their knowledge and skills, It also enables undergraduate students to carry out research projects

which is part of their university curriculum in order to graduate. This leads to gainful employment and less training is needed for the new employee. As part fostering collaboration between the Kenya Defense Forces (KDF) and KEMRI/USAMRU-K, the internship program also admits nominated KDF laboratory personnel for hands-on training in the GEIS laboratories. A total of 71 students completed internships under this program.

Port Reitz Hospital, like many hospitals in the developing world lacks many of the basic infrastructures necessary for a hospital its size. The laboratory is small and very crowded, it currently combines both routine sample testing and specialized tests in the same room. The lack of adequate space forces the laboratory staff to run different tests/screenings in the same area greatly increasing contamination risk and inaccurate laboratory results. These Major deficiencies can lead to duplicative and costly confirmatory testing to be done or for erroneous results to be submitted to authorities at the Ministry of Health, the hospital and others, potentially damaging USAMRU-K GEIS reputation. USAMRU-K GEIS is an important member of the Kenyan Public Health community, and played an active role during the YF outbreak in 2006 and the H1N1 pandemic in 2009. Renovations were completed and the building handed over to the Ministry of Health authorities.

KEY RESEARCH ACCOMPLISHMENTS:

Characterization of viral etiologies of influenza-like illness in Kenya

Characterization of viral etiologies of severe acute respiratory illness in Kenya

Identification of circulating strains of Influenza virus in Kenya

Characterization of selected viral, bacterial, and rickettsial etiologies of febrile illness in Kenya

Determination of leishmania prevalence in sand flies in select regions of Kenya

Response to dengue outbreak in coastal Kenya

Continued elucidation and tracking changes in antimalarial resistance patterns in Kenya

Identification of potential artemesinin resistance in Kenya

Training of Ministry of Health microscopists in accurate, reliable malaria microscopy

Ongoing characterization of etiologies of diarrheal illnesses in Kenya

Determination of prevalence of GC and Chlamydia among individuals seeking care with symptoms of STI

Human and infrastructure capacity development programs

REPORTABLE OUTCOMES: See references.

CONCLUSION:

KEMRI provides critical support to USAMRU-K's emerging infectious disease surveillance program in Kenya. Without KEMRI, USAMRU-K would not be able to execute its mission. KEMRI provides the legal and regulatory framework, personnel, and laboratory structure necessary to carry out scientific work. The organizations exist in partnership, with USAMRU-K working fully under the KEMRI umbrella in Kenya. Together, we have made great strides in establishing surveillance capabilities in the

areas of respiratory illnesses, acute febrile illnesses, malaria, enterics, sexually transmitted infections and antimicrobial resistance, and capacity building. KEMRI maintains both surveillance sites and central laboratories to accomplish this mission.

REFERENCES:

Respiratory:

Kiptinness JK, Wurapa EK, Wamunyokoli F, Bulimo WD. 2013. Molecular characterization of human parainfluenza virus type 1 in infants attending Mbagathi District Hospital, Nairobi, Kenya: a retrospective study. Virus genes.

Nzunza Rosemary, Wurapa Eyako, Kariuki Njenga, Chek John, Ongus Juliette, Bulimo Wallace. Epidemiological and Clinical Description of Human Metapneumovirus Infections in Mbagathi District Hospital, Kenya in 2008. OPTIONS for control of influenza Cape Town South Africa from 5th-10th September 2013.

Meshack Wadegu , Wallace D. Bulimo, Rachel A. Achilla, Janet Majanja, Silvanos Mukunzi, Finnley Osuna, Julia Wangui , Ken Mitei, Stephen Ocholla, Janet Nyambura, Josephat Mwangi, James Njiri , Benjamin Opot, Eyako K. Wurapa. Characterization of Neuraminidase Inhibitor Susceptibility of influenza A isolates obtained in Kenya, 2008-2011. OPTIONS for control of influenza Cape Town South Africa from 5th-10th September 2013.

Silvanos Mukunzi, Wallace D. Bulimo, Rachael A. Achilla, Meshack Wadegu, Janet Majanja, Benjamin Opot, Finnley Osuna, Keneth Kibet, James Njiri, Janet Muthoni, Stephen Ocholla, Josphat Mwangi and Eyako Wurapa. Genetic Variants of Influenza A (H1N1) Pdm09 Viruses That Circulated In Kenya During 2010 to 2011. OPTIONS for control of influenza Cape Town South Africa from 5th-10th September 2013.

Malaria:

David Saunders, Christopher Duplessis, Andres Lescano, Ben Andagalu, Alaina Thomas, Mark Fukuda, James Cummings. US Department of Defense contributions to malaria surveillance. The Lancet Infectious Diseases, Volume 13, Issue 4, Pages 293 - 294, April 2013

Misclassification of Plasmodium species by conventional microscopy and the impact of remedial training on the proficiency of laboratory technicians in species identification. Malaria Journal 2013, 12:113

Enterics:

Enteric pathogen surveillance in a case-control study of acute diarrhoea in the town of Kisii, Kenya, Swierczewski, B, Odundo, E, Koech, M, Ndonye, J, Kirera, R, Odhiambo, C, Cheriuyot, E, Shaffer, D, Ombogo, A, and Oaks, E. JMM Correspondence.

Comparison of the Triage Micro Parasite Panel and Microscopy for the Detection of Entamoeba histolytica/Entamoeba dispar, Giardia lamblia, and Cryptosporidium parvum in Stool Samples Collected in Kenya. Swierczewski, B, Odundo E, Ndonye, J, Kirera, J, Odhiambo, C, and Oaks, E. Journal of Tropical Medicine, vol. 2012.

Surveillance for enteric pathogens in a case-control study of acute diarrhea in western Kenya. Swierczewski, B.E., Odundo, E.A, Koech, M.C., Ndonye, J.N., Kirera,R.K., Odhiambo, C.P., Cheruiyot,E.K., Wu, M.T., Lee, J.E., Zhang, C. and Oaks, E.V. Trans R Soc Trop Med Hyg, 2012.

STI:

Alice Y. Tsai, Erica Dueger, Grace E. Macalino4, Silvia M. Montano, Margaret Mbuchi, Karen E. Saylors, Naiki Puplampu, Eric C. Garges, Samuel L. Yingst, R. Scott McClelland Olusegun O. Soge, Ann E. Jerse, Jose L. Sanchez. Neisseria gonorrhoeae (NG) Resistance Surveillance in Selected Populations of Five Countries IUSTI Congress Jul 2013, Vienna, Austria.

Sanchez T et al., 2011: U.S. Department of Defense (DoD) N. gonorrhoeae (GC) Global Resistance Surveillance Network. 12th IUSTI Conference, New Delhi India , 2011. 2.Tsai A. U. e.t al., : The Armed Forces Health Surveillance Centers Global STI Surveillance Program. Accepted; 2012 International Conference on Emerging Infectious Diseases (ICEID) in Atlanta, Georgia, USA, March 11 - 14, 2012 at the Hyatt Regency Atlanta